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Data in Brief

Transcriptome analysis of *Streptococcus pneumoniae* D39 in the presence of cobalt

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ABSTRACT

Cobalt (Co^{2+}) is an important transition metal ion that plays a vital role in cellular physiology of bacteria. The role of Co^{2+} in the regulation of several genes/operons in *Streptococcus pneumoniae* has recently been reported [1]. The data described in this article relate to the genome-wide transcriptional profiling of *Streptococcus pneumoniae* D39, either in the presence or absence of 0.5 mM Co^{2+} in chemically defined medium (CDM) using DNA microarray analysis. Genes belonging to a broad range of cellular processes such as virulence, transport and efflux systems, stress response and surface attachment were differentially expressed in the presence of Co^{2+}. We used transcriptional *lacZ* assays and electrophoretic mobility shift assays (EMSAs) to confirm our results [1]. The dataset is publicly available at the Gene Expression Omnibus (GEO) repository (http://www.ncbi.nlm.nih.gov/geo/) with accession number GSE57696.

1. Specifications

Organism/cell line/tissue	*Streptococcus pneumoniae* strain D39
Sex	N/A
Sequencer or array type	Oligo-based DNA microarray
Data format	Raw and processed
Experimental factors	0 mM Co^{2+} versus 0.5 mM Co^{2+}
Experimental features	Differentially expressed genes were identified by microarray comparison of D39 wild-type grown in CDM + 0 mM Co^{2+} to D39 wild-type grown in CDM + 0.5 mM Co^{2+} in CDM
Consent	N/A
Sample source location	Groningen, The Netherlands

2. Direct link to deposited data

The raw and processed DNA microarray dataset has been deposited in the Gene Expression Omnibus (GEO) database and can be accessed under following link: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE57696.

3. Experimental design, materials and methods

3.1. Objective of the experiment

Our objective was to investigate the impact of Co^{2+} on the gene expression of *S. pneumoniae*.

3.2. Strains and growth conditions

S. pneumoniae D39 serotype 2 strain (cps2), obtained from the laboratory of Prof. Peter Hermans, was used in this study [2]. The chemically defined medium (CDM) was treated with 1% Chelex 100 Resign (Bio-Rad) to ensure a metal depleted environment (medium). 50 ml of cell culture of *S. pneumoniae* D39 was grown in the CDMchelex either with or without 0.5 mM Co^{2+} at 37 °C in replicates. Cells were collected at an optical density of 0.2–0.25 (i.e. mid-exponential growth phase) at 600 nm (OD_{600}) by centrifugation for 1 min at 4 °C. The cell pellets were maintained at −80 °C if not processed immediately.

3.3. Total RNA extraction and removal of ribosomal RNA

Total RNA from the samples were isolated as described [3]. In short, cell pellets were resuspended in 400 μl of nuclease free water (DEPC-treated), after which 50 μl of 10% SDS, 500 μl of phenol/chloroform (1:1) and 500 mg glass beads were added and lysed by beat beater in the screw-capped tubes. Total RNA was isolated by the combination of

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the Macaloid method and the RNA isolation Kit (Roche) from lysed cells. DNA contamination was eliminated from the RNA sample by treatment with 2U of RNase free DNase I (Invitrogen, Paisley, United Kingdom). A NanoDrop Spectrophotometer (NanoDrop Technologies, Inc.) was used to determine the RNA concentration and sample quality was assessed using an Agilent RNA analysis kit (Agilent technologies).

3.4. cDNA preparation, hybridization and data acquisition

15 μg of RNA was mixed with 2 μl random nonamers (1.6 μg/μl) to prepare the annealing mixture. The volume of the annealing mixture was kept at 18 μl by the addition of nuclease free water (DEPC-treated), if required. The reaction mixture was kept at 70 °C for 5 min following 10 min cooling step at room temperature. 12 μl of master mix was prepared for each sample by the addition of 6 μl 5X first strand buffer [250 mM Tris–HCl (pH 8.3), 375 mM KCl, 15 mM MgCl2], 3 μl 0.1 M DDT, 1.2 μl 25X AA-dUTP/nucleotide mix, and 1.8 μl Superscript III reverse transcriptase. The master mix was added to the annealing mixture carefully, and incubated at 42 °C for 2–16 h. After incubation, the reaction mixture was treated with 3 μl of 2.5 M NaOH at 37 °C for 15 min to remove the mRNA from the reaction mixture. After that 15 μl of 2 M HEPES free acid was added in the reaction mixture to neutralize the NaOH. The cDNA mixture was purified by the DNA purification Kit (NucleoSpin, Gel and PCR clean-up kit), following the manufacturer's protocol. cDNA samples were labeled with DyLight-550 and DyLight-650 in dye-swap.

We combined the equal quantities of labeled cDNAs (max 30% difference), and dried the samples using the vacuum concentrators at high temperature (approx. 40 min) until the volume was smaller than 7 μl. The dried samples were dissolved in 7 μl H_2O and incubated at 94 °C for 2 min. Finally, hybridization was performed with labeled cDNA for 16 h at 45 °C in Ambion Slidehyb #1 hybridization buffer on in house build super amine glass slides (Array-It, SMMBC) containing amplicon of on average 600 bp representing 2087 ORFs of *S. pneumoniae* TIGR4 [4] and 184 ORFs specific for *S. pneumoniae* R6 [5]. 0.5 pmol/μl was taken as the minimum concentration of DyLight550 or DyLight650 in a total eluted volume of 50 μl. After hybridization, slides were washed using freshly prepared wash-buffers I, II and III and scanned at appropriate wavelengths in the scanner as described before [3].

3.5. Microarray data analysis

The microarray scanned slides were analyzed in GenePix Pro 6.0 Microarray Acquisition and by Analysis Software [6]. Raw data files were deposited on GEO under the accession number GSE57696. After initial analysis, the normalization and processing of the data was performed using different *Microprep* software package (Table 1). Statistical analyses were performed as described previously [7]. Finally, Cyber-T was used to analyze the data generated using *Microprep* for the identification of statistically significant differentially expressed genes. False discovery rates (FDRs) were calculated as described [8]. For differentially expressed genes, $p < 0.001$ and FDR < 0.05 were taken as a standard. Genes exhibiting a fold change ≥2.0 and a p-value < 0.05 were considered differentially expressed. Software packages mentioned in Table 1 were used for further data interpretation.

Table 2
Summary of downregulated genes in transcriptome comparison of *S. pneumoniae* D39 wild-type grown in CDM plus 0 mM Co^{2+} and CDM plus 0.5 mM Co^{2+}.

Gene tag[a]	Function[b]	Ratio[c]	P-value
SPD0053	Amidophosphoribosyltransferase	−2.0	2.09E-06
SPD0054	Phosphoribosylformylglycinamidine cyclo-ligase	−2.0	5.29E-08
SPD0055	Phosphoribosylglycinamide formyltransferase	−2.2	1.12E-05
SPD0056	VanZ protein	−2.4	6.93E-05
SPD0057	Bifunctional purine biosynthesis protein, PurH	−2.5	1.28E-07
SPD0187	Anaerobic ribonucleoside-triphosphate reductase, NrdD	−11.2	2.93E-14
SPD0188	Hypothetical protein	−4.3	2.47E-10
SPD0189	Acetyltransferase, GNAT family protein	−11.0	5.44E-10
SPD0190	Anaerobic ribonucleoside-triphosphate reductase, NrdG	−10.5	1.22E-15
SPD0191	Hypothetical protein	−8.3	1.34E-07
SPD0458	Heat-inducible transcription repressor, HrcA	−2.5	1.09E-10
SPD0459	Heat shock protein, GrpE	−2.1	4.82E-09
SPD0558	Cell wall-associated serine protease, PrtA	−16.6	3.89E-14
SPD1461	Mn^{2+} ABC transporter, ATP binding protein, PsaB	−8.7	2.18E-14
SPD1462	Manganese ABC transporter, permease protein, PsaC	−8.7	2.18E-14
SPD1594	XRE family Transcriptional regulator	−3.1	7.14E-09
SPD1636	Zn^{2+}-containing alcohol dehydrogenase	−8.4	2.05E-14
SPD1637	MerR family transcriptional regulator	−11.0	1.41E-10
SPD1638	Cation efflux system, CzcD	−20.8	0
SPD1965	Choline binding protein, PcpA	−5.0	5.48E-06
SPD2044	Rod shape-determining protein, MreD	−3.0	8.75E-11
SPD2046	Co^{2+} ABC transporter, permease protein, CbiQ	−2.0	1.22E-09
SPD2049	CDP-diacylglycerol-glycerol-3-phosphate 3-phosphatidyltransferase PgsA	−2.0	1.13E-06
SPD2052	Hypothetical protein	−2.0	6.84E-09

[a] Gene numbers refer to D39 locus tags.
[b] D39 annotation/TIGR4 annotation [5,13].
[c] Ratios (0 mM Co^{2+}/0.5 mM Co^{2+}).

4. Discussion

Here, we have investigated the impact of Co^{2+} on the global gene expression of *S. pneumoniae* D39 by DNA microarray analysis. Transcriptome comparison of D39 wild-type grown in CDM with 0 mM Co^{2+} to same strain grown in CDM with 0.5 mM Co^{2+}, revealed the impact of Co^{2+} on the gene expression of *S. pneumoniae* D39. 24 genes were downregulated (Table 2) and 14 genes were upregulated (Table 3). The PsaR regulon (*pcpA*, *psaBCA* and *prtA*), the *cbi* operon, and the *nrd* operon were highly downregulated in the absence of Co^{2+}, suggesting the role of Co^{2+} in the regulation of these systems. This was further confirmed by β-galactosidase assays, metal accumulation assays and electrophoretic mobility shift assays (EMSAs) [1]. The expression of some other genes was also altered in our transcriptome analysis and further investigations are required to clear the role of Co^{2+} in the regulation of these genes.

Table 1
Summary of computational tools used to analyze DNA microarray data.

Software	Purpose	URL
Microprep [8]	A cDNA microarray data pre-processing framework	http://www.molgenrug.nl/index.php/molgensoftware
CyberT	Amplementation of a variant of *t-test*	http://bioinformatics.biol.rug.nl/cybert/index.shtml
Genome2D [9]	A visualization tool for the rapid analysis of bacterial transcriptome data	http://genome2d.molgenrug.nl/
FIVA [10]	Functional Information Viewer and Analyzer extracting biological knowledge from transcriptome data of prokaryotes	http://bioinformatics.biol.rug.nl/standalone/fiva/
Projector [11]	Automatic contig mapping for gap closure purposes	http://bamics2.cmbi.ru.nl/websoftware/projector2/projector2_start.php
PePPER [12]	A webserver for prediction of prokaryote promoter elements and regulons	http://pepper.molgenrug.nl/

Table 3
Summary of upregulated genes in transcriptome comparison of *S. pneumoniae* D39 wild-type grown in CDM plus 0 mM Co^{2+} and CDM plus 0.5 mM Co^{2+}.

Gene tag[a]	Function[b]	Ratio[c]	P-value
SPD0801	Hypothetical protein	2.01	1.29E-05
SPD0910	Serine hydroxymethyltransferase	2.17	1.69E-11
SPD1018	Immunoglobulin A1 protease precursor	2.08	2.29E-06
SPD1039	Phosphoenolpyruvate-protein phosphotransferase	2.05	5.54E-11
SPD1053	Galactose-6-phosphate isomerase, LacA subunit	2.45	5.83E-07
SPD1294	Hypothetical protein	2.65	1.98E-11
SPD1355	Hypothetical protein	2.10	7.71E-01
SPD1466	ABC transporter, ATP-binding protein	2.02	4.65E-09
SPD1588	Hypothetical protein	2.29	3.09E-07
SPD 1598	Hypothetical protein	2.16	2.62E-08
SPD 1596	Tryptophan synthase, alpha subunit	2.24	2.36E-08
SPD 1727	Hypothetical protein	2.39	2.61E-07
SPD 1728	Hypothetical protein	2.26	1.25E-10

[a] Gene numbers refer to D39 locus tags.
[b] D39 annotation/TIGR4 annotation [5,13].
[c] Ratios (0 mM Co^{2+}/0.5 mM Co^{2+}).

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